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(54) **METHODE DE FABRICATION ET/OU DE REPARATION DE CARTILAGE**

(54) **METHOD FOR MAKING AND/OR REPAIRING CARTILAGE**

(57) La présente invention concerne des méthodes de fabrication et/ou de réparation de cartilage in vivo comportant l'implantation, dans un site où le cartilage est endommagé ou perdu, d'un support ou structure tridimensionnelle biocompatible et non vivante en association avec du tissu périostatique/périchondral qui peut être utilisé pour tenir le support en place et servir de source de cellules souches de chondrocytes, de chondrocytes et d'autres cellules du stroma à fixer au support in vivo. Une préparation de cellules pouvant contenir des chondrocytes, des cellules souches de chondrocytes ou d'autres cellules du stroma est de plus administrée, soit avant, pendant ou après la greffe du support et/ou du tissu périostique/périchondral; les cellules sont administrées directement dans le site de l'implant in vivo et favorisent l'induction de facteurs qui accélèrent la chondrogenèse et la migration de chondrocytes, de cellules souches et d'autres cellules du stroma du milieu adjacent in vivo vers le support pour la production de nouveau cartilage au site de la greffe.

(57) The present invention relates to methods of making and/or repairing cartilage in vivo comprising implanting into a patient, at a site of cartilage damage or loss, a biocompatible, non-living three-dimensional scaffold or framework structure in combination with periosteal/perichondrial tissue that can be used to hold the scaffold in place and provides a source of chondrocyte progenitor cells, chondrocytes and other stromal cells for attachment to the scaffold in vivo. In addition, a preparation of cells that can include chondrocytes, chondrocyte progenitor cells or other stromal cells is administered, either before, during or after implantation of the scaffold and/or the periosteal perichondrial tissue; the cells are administered directly into the site of the implant in vivo and promote the induction of factors that enhance chondrogenesis and the migration of chondrocytes, progenitor cells and other stromal cells from the adjacent in vivo environment into the scaffold for the production of new cartilage at the site of implantation.

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(54) Title: METHOD FOR MAKING AND/OR REPAIRING CARTILAGE

(57) Abstract

The present invention relates to methods of making and/or repairing cartilage *in vivo* comprising implanting into a patient, at a site of cartilage damage or loss, a biocompatible, non-living three-dimensional scaffold or framework structure in combination with periosteal/perichondrial tissue that can be used to hold the scaffold in place and provides a source of chondrocyte progenitor cells, chondrocytes and other stromal cells for attachment to the scaffold *in vivo*. In addition, a preparation of cells that can include chondrocytes, chondrocyte progenitor cells or other stromal cells is administered, either before, during or after implantation of the scaffold and/or the periosteal perichondrial tissue; the cells are administered directly into the site of the implant *in vivo* and promote the induction of factors that enhance chondrogenesis and the migration of chondrocytes, progenitor cells and other stromal cells from the adjacent *in vivo* environment into the scaffold for the production of new cartilage at the site of implantation.

AMENDED CLAIMS

[received by the International Bureau on 9 July 1997 (09.07.97); original claims 1-46 replaced by new claims 1-47 (8 pages)]

1. The use of a biocompatible, non-living three-dimensional scaffold structure in combination with periosteal tissue, perichondrial tissue or a combination of said tissues in conjunction with a preparation of stromal cells for the production, subsequent to implantation, of cartilage.

2. The use according to claim 1, characterized by the fact that said stromal cells attach to the scaffold and/or induce chondrogenesis or migration of stromal cells.

3. The use according to claim 1 or 2, characterized by the fact that the periosteal or perichondrial tissue is located on top or at the bottom of and adjacent to the scaffold.

4. The use according to claim 1 or 2, characterized by the fact that the periosteal or perichondrial tissue is situated with respect to the scaffold such that stromal cells from the tissue can migrate from the tissue to the scaffold.

5. The use according to claim 1 or 2, characterized by the fact that the periosteal or perichondrial tissue is situated with respect to the scaffold such that the cambium or inner transition layers of the tissue faces the scaffold.

6. The use according to claim 1 or 2, characterized by the fact that the preparation of stromal cells is physically placed between the scaffold and the periosteal or perichondrial tissue.

7. The use according to any one of claims 1 to 6, characterized by the fact that the scaffold structure is composed of a biodegradable or non-biodegradable material.

8. The use according to claim 7, characterized by the fact that the biodegradable material is polyglycolic acid, polylactic acid, cotton, cat gut sutures, cellulose, nitrocellulose, gelatin, collagen or polyhydroxyalkanoates.

9. The use according to claim 7, characterized by the fact that the non-biodegradable material is a polyamide, a polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a polytetrafluorethylene, thermanox, cotton, cellulose or a polyhydroxyalkanoate.

10. The use according to any one of claims 1 to 9, characterized by the fact that the framework is a felt or a mesh.

11. The use according to any one of claims 1 to 10, characterized by the fact that the scaffold is treated with ethylene oxide or with an electron beam prior to implantation.

12. The use according to any one of claims 1 to 11, characterized by the fact that the scaffold comprises or is modified to contain at least one substance capable of enhancing the attachment or growth of stromal cells on the scaffold.

13. The use according to claim 12, characterized by the fact that the substance is (a) a bioactive agent selected from the group consisting of cellular growth factors, factors that stimulate migration of stromal cells, factors that stimulate chondrogenesis, factors that stimulate matrix deposition, anti-inflammatories, and immunosuppressants, or (b) is selected from the group consisting of collagens, elastic fibers, reticular fibers, heparin sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, keratin sulfate and hyaluronic acid.

14. The use according to claim 13, characterized by the fact that the bioactive agent (a) is a TGF-β, with or without ascorbate, or (b) is a bone morphogenetic protein that stimulates cartilage formation, or (c) comprises a sustained release formulation.

15. The use according to claim 14, characterized by the fact that the formulation comprises a composite of the bioactive agent and a biocompatible polymer.

16. The use according to claim 15, characterized by the fact that the biocompatible polymer is selected from the group consisting of polylactic acid, poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, and collagen.

17. The use according to any one of claims 1 to 16, characterized by the fact that the preparation of stromal cells comprises chondrocytes, or chondrocyte progenitor cells, or fibroblasts or fibroblast-like cells, or a combination of chondrocytes, chondrocyte progenitor cells, fibroblasts, fibroblast-like cells, endothelial cells, pericytes, macrophages, monocytes, leukocytes, plasma cells, mast cells, adipocytes, umbilical cord cells, or bone marrow cells from umbilical cord blood.

18. The use according to claim 17, characterized by the fact that the chondrocyte progenitor cells comprise mesenchymal stem cells.

19. The use according to any one of claims 1 to 18, characterized by the fact that the preparation of stromal cells further comprise at least one bioactive agent or said cells are genetically engineered to produce at least one bioactive agent.

20. The use according to claim 19, characterized by the fact that the bioactive agent is selected from the group

consisting of cellular growth factors, factors that stimulate migration of stromal cells, factors that stimulate chondrogenesis, factors that stimulate matrix deposition, anti-inflammatories, and immunosuppressants.

21. The use according to claim 20, characterized by the fact that the bioactive agent is a TGF-β, with or without ascorbate, or a bone morphogenetic protein that stimulates cartilage formation.

22. The use according to any one of claims 1 to 18, characterized by the fact that the stromal cells of the preparation are genetically engineered to express a gene that is deficiently expressed in vivo, or to prevent or reduce the expression of a gene normally expressed by the stromal cells.

23. A method of producing cartilage at a cartilage defect site in vivo comprising:

(a) implanting into the defect site a biocompatible, non-living three-dimensional scaffold structure in combination with periosteal tissue, perichondrial tissue or a combination of said tissues; and

(b) additionally administering into the defect site a preparation of stromal cells for attachment to the scaffold and/or for inducing chondrogenesis or migration of stromal cells from the in vivo environment adjacent to the defect site to the scaffold.

24. The method of claim 1, wherein the scaffold is implanted into the defect site and the periosteal or perichondrial tissue is placed on top of and adjacent to the scaffold.

25. The method of claim 1, wherein the periosteal or perichondrial tissue is implanted into the defect site and the scaffold is placed on top of and adjacent to the tissue.

26. The method of claim 1, wherein the periosteal or perichondrial tissue is situated with respect to the scaffold such that stromal cells from the tissue can migrate from the tissue to the scaffold.

27. The method of claim 1, wherein the periosteal tissue is situated with respect to the scaffold such that the cambium layer of the tissue faces the scaffold.

28. The method of claim 1, wherein the perichondrial tissue is situated with respect to the scaffold such that the cambium or inner transition layers of the tissue face the scaffold.

29. The method of claim 1, wherein the preparation of stromal cells is administered prior to, during or after implantation of the scaffold structure.

30. The method of claim 1, wherein the preparation of stromal cells is administered prior to, during or after implantation of the periosteal or perichondrial tissue.

31. The method of claim 1, wherein the preparation of stromal cells is physically placed between the scaffold and the periosteal or perichondrial tissue.

32. The method according to any one of claims 23 to 31, characterized by the fact that the scaffold structure is composed of a biodegradable or a non-biodegradable material.

33. The method according to claim 32, characterized by the fact that the biodegradable material is polyglycolic acid, polylactic acid, cotton, cat gut sutures, cellulose, nitrocellulose, gelatin, collagen or polyhydroxyalkanoates.

34. The method according to claim 32, characterized by the fact that the non-biodegradable material is a polyamide,

a polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a polytetrafluorethylene, thermanox, cotton, cellulose or a polyhydroxyalkanoate.

35. The method according to any one of claims 23 to 34, characterized by the fact that the framework is a felt or a mesh.

36. The method according to any one of claims 23 to 35, characterized by the fact that the scaffold is treated with ethylene oxide or with an electron beam prior to implantation.

37. The method according to any one of claims 23 to 36, characterized by the fact that the scaffold comprises or is modified to contain at least one substance capable of enhancing the attachment or growth of stromal cells on the scaffold.

38. The method according to claim 37, characterized by the fact that the substance is (a) a bioactive agent selected from the group consisting of cellular growth factors, factors that stimulate migration of stromal cells, factors that stimulate chondrogenesis, factors that stimulate matrix deposition, anti-inflammatories, and immunosuppressants, or (b) is selected from the group consisting of collagens, elastic fibers, reticular fibers, heparin sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, keratin sulfate and hyaluronic acid.

39. The method according to claim 38, characterized by the fact that the bioactive agent (a) is a TGF-β, with or without ascorbate, or (b) is a bone morphogenetic protein that stimulates cartilage formation, or (c) comprises a sustained release formulation.

40. The method according to claim 39, characterized by the fact that the formulation comprises a composite of the bioactive agent and a biocompatible polymer.

41. The method according to claim 40, characterized by the fact that the biocompatible polymer is selected from the group consisting of polylactic acid, poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, and collagen.

42. The method according to any one of claims 23 to 41, characterized by the fact that the preparation of stromal cells comprises chondrocytes, or chondrocyte progenitor cells, or fibroblasts or fibroblast-like cells, or a combination of chondrocytes, chondrocyte progenitor cells, fibroblasts, fibroblast-like cells, endothelial cells, pericytes, macrophages, monocytes, leukocytes, plasma cells, mast cells, adipocytes, umbilical cord cells, or bone marrow cells from umbilical cord blood.

43. The method according to claim 42, characterized by the fact that the chondrocyte progenitor cells comprise mesenchymal stem cells.

44. The method according to any one of claims 23 to 43, characterized by the fact that the preparation of stromal cells further comprise at least one bioactive agent or said cells are genetically engineered to produce at least one bioactive agent.

45. The method according to claim 44, characterized by the fact that the bioactive agent is selected from the group consisting of cellular growth factors, factors that stimulate migration of stromal cells, factors that stimulate chondrogenesis, factors that stimulate matrix deposition, anti-inflammatories, and immunosuppressants.

46. The method according to claim 45, characterized by the fact that the bioactive agent is a TGF-β, with or without ascorbate, or a bone morphogenetic protein that stimulates cartilage formation.

47. The method according to any one of claims 23 to 43, characterized by the fact that the stromal cells of the preparation are genetically engineered to express a gene that is deficiently expressed in vivo, or to prevent or reduce the expression of a gene normally expressed by the stromal cells.

METHOD FOR MAKING AND/OR REPAIRING CARTILAGE

1. INTRODUCTION

The present invention relates to methods for making and/or repairing cartilage in vivo. More specifically, the invention relates to methods of making and/or repairing cartilage comprising implanting into a patient, at a site of cartilage damage or loss, a biocompatible, non-living three-dimensional scaffold or framework structure, in combination with periosteal/perichondrial tissue, and administering a preparation of chondrocytes and/or other stromal cells, such as chondrocyte progenitor cells, to the site of the implant before, during or after implantation of the scaffold and/or the periosteal/perichondrial tissue. The periosteal/perichondrial tissue can be used to hold the scaffold in place at the site of implantation and also provides a source of stromal cells, e.g., chondrocytes and/or chondrocyte progenitor cells, for attachment to the scaffold in vivo. The preparation of stromal cells seeded directly into the implantation site in vivo provides not only a readily-accessible source of chondrocytes and/or other stromal cells for attachment to the scaffold but also provides a rapid and efficient means of inducing chondrogenesis as well as migration of stromal cells from the surrounding in vivo environment to the scaffold via factors produced by the stromal cells of the preparation. Additionally, the seeded stromal cells can be genetically engineered to express gene products beneficial to growth, implantation and/or amelioration of disease conditions. The methods of the invention therefore result in the efficient production of new cartilage in vivo.

The methods of this invention are useful in the production/repair of articular cartilage in patients suffering from degenerative connective tissue diseases such as rheumatoid and/or osteoarthritis as well as in patients who have cartilage defects due to trauma. The methods of this invention can be used to replace or augment existing

cartilage tissue, to introduce new or altered tissue or to join together biological tissues or structures.

2. BACKGROUND OF THE INVENTION

There are various types of cartilage, e.g., articular or hyaline cartilage, elastic cartilage and fibrocartilage. Articular cartilage is found at the articular surfaces of bones, e.g., in the joints, and is responsible for providing the smooth gliding motion characteristic of moveable joints. Articular cartilage is firmly attached to the underlying bones and measures less than 5mm in thickness in human joints, with considerable variation depending on joint and site within the joint. In addition, articular cartilage is aneural, avascular, and alymphatic. In adult humans, this cartilage derives its nutrition by a double diffusion system through the synovial membrane and through the dense matrix of the cartilage to reach the chondrocyte, the cells that are found in the connective tissue of cartilage.

In fact, articular cartilage consists of highly specialized chondrocyte cells surrounded by a dense extracellular matrix consisting mainly of type II collagen, proteoglycan and water. While the biochemical composition of articular cartilage includes up to 65-80% water (depending on the cartilage), the collagen component of the cartilage is the most prevalent organic constituent. The collagen (mainly type II) accounts for about 15-25% of the wet weight or about half the dry weight, except in the superficial zone where it accounts for most of the dry weight. Its concentration is usually progressively reduced with increasing depth from the articular surface. The proteoglycan content accounts for up to 10% of the wet weight or about a quarter of the dry weight. Proteoglycans consist of a protein core to which linear sulfated polysaccharides are attached, mostly in the form of chondroitin sulfate and keratin sulfate. In addition to type II collagen, articular collagen contains several other collagen types (IV, V, IX and X) with distinct structures. There are a variety of interactions between

these individual macromolecules, which include both noncovalent associations between proteoglycans and collagens, and covalent bonds between different collagen species. Resistance of the extracellular matrix to water flow gives cartilage its ability to dispense high joint loads. It absorbs shock and minimizes stress on subchondral bone (Mow et al., 1984, J. Biomech. 17:377-394). However, adult cartilage and bone have a limited ability of repair; thus, damage to cartilage produced by disease, such as rheumatoid and/or osteoarthritis, or trauma can lead to serious physical deformity and debilitation. Furthermore, as human articular cartilage ages, its tensile properties change. The superficial zone of the knee articular cartilage exhibits an increase in tensile strength up to the third decade of life, after which it decreases markedly with age as detectable damage to type II collagen occurs at the articular surface. The deep zone cartilage also exhibits a progressive decrease in tensile strength with increasing age, although collagen content does not decrease. These observations indicate that there are changes in mechanical and, hence, structural organization of cartilage with aging that, if sufficiently developed, can predispose cartilage to traumatic damage. In osteoarthritic cartilage, there is excessive damage to type II collagen, resulting in crimping of collagen fibrils. In rheumatoid arthritis, the combined actions of free radicals and proteinases released from polymorpholeukocytes cause much of the damage seen at the articular surface (Tiku et al., 1990, J. Immunol. 145:690-696). Induction of cartilage matrix degradation and proteinases by chondrocytes is probably induced primarily by interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α) (Tyler, 1985, Biochem. J. 225:493-507).

The current therapy for damage or loss of cartilage is replacement with a prosthetic material, for example, silicone for cosmetic repairs, or metal alloys for joint realignment. Implantation of prosthetic devices is usually associated with loss of underlying tissue and bone without recovery of the

full function allowed by the original cartilage. Serious long-term complications associated with the presence of a permanent foreign body can include infection, erosion and instability.

Use of sterilized bone or bone powder or surgical steel seeded with bone cells that are eventually implanted have been largely unsuccessful because of the non-degradable nature of the cell support. According to one procedure, fibroblasts are exposed in vitro for a minimum of three days to a soluble bone protein capable of stimulating a chondrogenic response. The activated fibroblasts are then transferred in vivo by combining them with a biodegradable matrix or by intra-articular injection or attachment to allografts or prosthetic devices. The disadvantage of this method is that chondrogenesis is not allowed to develop in the short-term cultures and there is an unduly heavy reliance on cartilage synthesis by the exposed fibroblasts at the implant site. See Caplan, A., United States Pat. No. 4,609,551, issued September 2, 1986.

United States Pat. No. 5,041,138 to J.P. Vacanti et al., issued August 20, 1991, describes the growth of cartilaginous structures by seeding chondrocytes on biodegradable matrices in vitro for subsequent implantation in vivo. United States Patents Nos. 5,197,985 and 5,226,914, to Caplan et al., issued March 30, 1993 and July 13, 1993, respectively, relate to culturing marrow-derived mesenchymal stem cells in vitro in the presence of growth factors, applying these cells to a carrier, e.g., a porous ceramic vehicle, to promote round cell morphology, and implanting the carrier containing the cells into damaged articular cartilage. Finally, United States Pat. No. 4,520,821, to Schmidt et al., issued June 4, 1985 relates to a method of growing a biological tissue correction structure using a bioabsorbable mesh or gauze. According to a preferred embodiment, biological tissue is removed from a defective area of the body and grown in vitro on a bioabsorbable mesh and the tissue/mesh structure is placed into the area of the defect in vivo for a time long

enough to allow the mesh to be completely bioabsorbed. In all of the approaches cited above, cells are applied to a carrier in vitro for implantation into the site of a cartilage lesion.

United States Pat. No. 4,846,835 to Grande et al., issued July 11, 1989, also describes a grafting technique for promoting articular cartilage healing by culturing autologous chondrocytes in vitro and seeding the chondrocytes on a collagen matrix in vitro. Grande further describes implanting the chondrocyte/collagen matrix into a cartilage lesion and mechanically fixing the graft by suturing a periosteal flap to the cartilage. Grande teaches that the periosteum has very little chondrogenic potential and that the periosteal flap utilized therein could have been substituted by a resorbable biocompatible polymer to achieve the same fixation purpose. While von Schroeder et al., 1991, J. Biomed. Mat. Res. 25: 329-339, relates to periosteal grafts in combination with a polylactic acid matrix for the repair of full-thickness osteochondral defects in animals, Messner, 1994, Biomaterials 15 (No. 3): 223-230 describes experiments in which periosteal grafts in combination with Teflon and Dacron felts for the repair of full-thickness osteochondral defects were unsatisfactory in achieving normal cartilage repair in animals. O'Driscoll et al., 1986, J. Bone and Joint Surg. 68-A (No. 7): 1017-1035; O'Driscoll et al., 1985, 31st Annual ORS, p. 292, Las Vegas, Nevada, Jan. 21-24, 1985; Rubak, 1982, Acta. Orthop. Scand. 53: 175-180; and Messner et al., 1993, Biomaterials 14 (No. 7): 513-521 relate to the use of free autogenous periosteal grafts that are placed directly into full-thickness articular defects for the repair of such defects in animals.

In addition, Thoma et al., 1993, Plast. Reconstr. Surg. 91 (No. 2): 307-315 and Bruns et al., 1992, Virchows. Arch. A. Pathol. Anat. Histopathol. 421 (No. 1): 1-8 relate to the use of autogenous perichondrial grafts for the repair of full-thickness articular defects in animals. Thoma noted marked degenerative changes resembling osteoarthritis in the

perichondrial grafts and concluded that spontaneous repair of such large defects may result in a more normal new articular cartilage than the perichondrial grafts attempted. Bruns noted hyaline-like cartilage in non-weight-bearing areas of the defect, but not in weight-bearing areas. Finally, Bean et al., 1994, ORL J. Otorhinolaryngol. Relat. Spec. 56: 224-229 reports the use of a composite graft of demineralized bovine bone matrix and autogenous perichondrium for the reconstruction of the anterior laryngeal wall in rabbits.

3. SUMMARY OF THE INVENTION

The present invention relates to methods of making and/or repairing cartilage in vivo comprising implanting into a patient, at a site of cartilage damage or loss, a biocompatible, non-living three-dimensional scaffold or framework structure in combination with periosteal/perichondrial tissue that can be used to hold the scaffold in place and provides a source of chondrocyte progenitor cells, chondrocytes and other stromal cells for attachment to the scaffold in vivo. In addition, a preparation of cells that can include chondrocytes, chondrocyte progenitor cells or other stromal cells is administered, either before, during or after implantation of the scaffold and/or the periosteal/perichondrial tissue; the cells are administered directly into the site of the implant in vivo and promote chondrogenesis and the production of factors that induce the migration of chondrocytes, progenitor cells and other stromal cells from the adjacent in vivo environment into the scaffold for the production of new cartilage at the site of implantation.

More specifically, the three-dimensional scaffold contains interstitial spaces into which progenitor cells, chondrocytes and other stromal cells from the adjacent in vivo environment, including the implanted periosteal/perichondrial tissue, migrate for attachment and growth on and within the scaffold structure. The preparation of stromal cells seeded in combination with the scaffold and

periosteal/perichondrial tissue provides a ready source of chondrocytes and other stromal cells which produce biological factors that promote chondrogenesis and the migration of stromal cells from, e.g., the periosteal/perichondrial tissue to the scaffold for attachment and/or differentiation thereon. The stromal cell preparation also provides a direct source of stromal cells, e.g., chondrocytes and/or progenitor cells, that are capable of migrating into the scaffold and attaching thereto. The stromal cells in the scaffold, whether derived from the periosteal/perichondrial tissue, from the exogenous stromal cell preparation or from the in vivo environment adjacent to the implant site, grow on the scaffold to form a cellular matrix and provide the support, growth factors and regulatory factors required for cartilage formation at a cartilage defect site in vivo. The methods of this invention thus result in the production of new cartilage in vivo at the implant site.

In a preferred embodiment of the methods of the invention, the periosteal/perichondrial tissue is placed over the implanted scaffold at the site of cartilage damage or loss ("the defect site") and affixed, e.g., by sutures, to that site, thus holding the scaffold in place. According to a further preferred embodiment, the scaffold is composed of a biodegradable material such that, upon successful engraftment, the scaffold structure is completely absorbed in vivo, resulting in new cartilage having no foreign, non-living material encompassed within it.

According to another preferred embodiment, the preparation of chondrocytes and/or other stromal cells is administered in vivo to the site of the implant after the scaffold and periosteal/perichondrial tissue have been implanted. In yet a further embodiment of the invention, bioactive agents such as cellular growth factors (e.g., TGF-β), factors that stimulate chondrogenesis (e.g., bone morphogenic proteins (BMPs) that promote cartilage formation), factors that stimulate migration of stromal cells and/or matrix deposition, anti-inflammatories or

immunosuppressants, are included at the implantation site. For example, these factors can be incorporated into the scaffold material to provide for release at the site of implantation; the scaffold can also be comprised of, or coated with, one or more of these bioactive agents. Alternatively, the factor(s) can be administered into or adjacent to the scaffold, either before, during or after seeding of the stromal cells, e.g., the bioactive agent(s) can be administered to the site, either as a separate preparation or as part of the stromal cell preparation. In addition, the stromal cells seeded at the defect site can be genetically engineered to express the genes for these bioactive agents, e.g., specific types of TGF-β such as TGF-β1 or specific types of BMPs such as BMP-13. Exposure of the defect site to these bioactive agents promotes the successful and/or improved production of new cartilage and/or improves the success of implantation, for example, by reducing the risk of rejection or inflammation associated with the implant.

For example, according to one embodiment of the invention, the stromal cells can be genetically engineered to express anti-inflammatory gene products to ameliorate the effects of degenerative diseases like rheumatoid arthritis which result in cartilage damage due to inflammatory reactions; e.g., the stromal cells can be engineered to express peptides or polypeptides corresponding to the idiotype of neutralizing antibodies for granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), interleukin-2 (IL-2), or other inflammatory cytokines and mediators.

According to another embodiment, the stromal cells can be genetically engineered to express tissue factors that enhance migration of stromal cells from the adjacent in vivo environment into the scaffold at the implantation site.

Preferably, the cells are engineered to express such gene products transiently and/or under inducible control during the post-operative recovery period, or as a chimeric

fusion protein anchored to the stromal cell, e.g., a chimeric molecule composed of an intracellular and/or transmembrane domain of a receptor or receptor-like molecule, fused to the gene product as the extracellular domain.

In another alternative embodiment, the stromal cells can be genetically engineered to "knock out" expression of factors that promote rejection of the implant or degenerative changes in articular cartilage due to aging, rheumatoid disease or inflammation. For example, expression of pro-inflammatory mediators such as GM-CSF, TNF, IL-1, IL-2 and cytokines can be knocked out in the exogenously-administered stromal cells or on the implanted periosteal or perichondrial tissue to reduce the risk of inflammation. Likewise, the expression of MHC class II molecules on the cells or tissues can be knocked out in order to reduce the risk of rejection of the implant.

In another embodiment of the invention, the methods of the invention may afford a vehicle for introducing genes and gene products <u>in vivo</u> to assist or improve the results of the implantation and/or for use in gene therapies. For example, genes that prevent or ameliorate symptoms of degenerative changes in cartilage such as rheumatoid disease or inflammatory reactions and bone resorption, may be underexpressed or overexpressed in disease conditions and/or due to aging. Thus, the level of gene activity in the patient may be increased or decreased, respectively, by gene replacement therapy by adjusting the level of the active gene product in genetically engineered stromal cells.

In yet another preferred embodiment, the cartilage defect site into which the implant will be placed is treated, preferably prior to implantation, to degrade the pre-existing cartilage at the defect site, freeing cells to migrate into the scaffold of the implant and promoting the orderly deposition of new cartilage. Methods of such treatment include enzymatic treatment, abrasion or microdrilling. According to a further embodiment, the preparation of stromal cells of the invention can be injected into the degraded

cartilage at the defect site, e.g., into the surrounding cells or into the walls of the defect, providing a source of biological factors that induce migration of stromal cells from the degraded cartilage to the implant.

4. DETAILED DESCRIPTION OF THE INVENTION

The present invention involves methods of making and/or repairing cartilage *in vivo* comprising the implantation *in vivo* of a three-dimensional scaffold or framework structure made of a biocompatible, non-living material in combination with periosteal/perichondrial tissue and the administration of a preparation of stromal cells, such as chondrocytes or chondrocyte progenitor cells, which cells are seeded at the site of implantation *in vivo*.

The term "chondrocyte progenitor cell" as used herein refers to either (1) a pluripotent, or lineage-uncommitted, progenitor cell, typically referred to in the art as a "stem cell" or "mesenchymal stem cell", which is potentially capable of an unlimited number of mitotic divisions to either renew its line or to produce progeny cells which will differentiate into chondrocytes; or (2) a lineage-committed progenitor cell produced from the mitotic division of a stem cell which will eventually differentiate into a chondrocyte. Unlike the stem cell from which it is derived, the lineage-committed progenitor is generally considered to be incapable of an unlimited number of mitotic divisions and will eventually differentiate into a chondrocyte.

The term "cartilage" or "cartilage tissue" as used herein is generally recognized in the art, and refers to a specialized type of dense connective tissue comprising cells embedded in an extracellular matrix (ECM) (see, for example, Cormack, 1987, *Ham's Histology*, 9th Ed., J.B. Lippincott Co., pp. 266-272). The biochemical composition of cartilage differs according to type; however, the general composition of cartilage comprises chondrocytes surrounded by a dense ECM consisting of collagen, proteoglycans and water. Several types of cartilage are recognized in the art, including, for

example, hyaline or articular cartilage such as that found within the joints, fibrous cartilage such as that found within the meniscus and costal regions, and elastic cartilage. The production of any type of cartilage is intended to fall within the scope of the invention.

Furthermore, although the invention is directed predominantly to methods for the production of new cartilage tissue in humans, the invention may also be practiced so as to produce new cartilage tissue in any mammal in need thereof, including horses, dogs, cats, sheep, pigs, among others. The treatment of such animals is intended to fall within the scope of the invention.

The invention is divided into the following sections solely for the purpose of description: (a) the three-dimensional scaffold; (b) the periosteal/perichondrial tissue and its implantation in combination with the scaffold; (c) the stromal cell preparation, including genetically engineered stromal cells; (d) administration of the stromal cells in vivo and (e) uses of the methods of the invention.

4.1. THE THREE-DIMENSIONAL SCAFFOLD

The three-dimensional scaffold or framework structure may be of any material and/or shape that: (a) allows cells to attach to it (or can be modified to allow the cells to attach to it); and (b) allows cells to grow in more than one layer. Because the three-dimensional structure is to be implanted in vivo, it may be preferable to use biodegradable materials such as polyglycolic acid (PGA), polylactic acid (PLA), hyaluronic acid, catgut suture material, gelatin, cellulose, nitrocellulose, collagen, cotton, or other naturally-occurring biodegradable materials. Furthermore, it may be preferable to sterilize the three-dimensional structure prior to implantation, e.g., by treatment with ethylene oxide or by gamma irradiation or irradiation with an electron beam.

In addition, a number of other materials may be used to form the scaffold or framework structure, including but not limited to: nylon (polyamides), dacron (polyesters),

polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluorethylene (PTFE, teflon), thermanox (TPX), and a variety of polyhydroxyalkanoates. Because certain of these materials, such as nylon, polystyrene, etc. may be poor substrates for cellular attachment, when these materials are used as the three-dimensional framework, it is advisable to pre-treat the framework prior to implantation in order to enhance the attachment of chondrocytes and other stromal cells to the scaffold. For example, nylon matrices can be treated with 0.1 M acetic acid and incubated in polylysine, PBS, and/or collagen to coat the nylon. Polystyrene could be similarly treated using sulfuric acid.

Any of the above-listed materials may be formed into a mesh or a felt, for example, to produce the three-dimensional framework or scaffold for use in the methods of this invention. Regardless of the shape of the framework, the openings of the framework should be of an appropriate size to allow the chondrocytes and other stromal cells to stretch across the openings. Maintaining actively growing stromal cells which stretch across the framework enhances the production of growth factors which are elaborated by the stromal cells, and further enhances new cartilage formation in vivo. In addition, the openings of the framework must allow for adequate diffusion of nutrients and waste products into and out of the structure and for vascularization at the site of implantation. For example, if the openings are too small, the stromal cells may rapidly achieve confluence but be unable to easily exit from the matrix; trapped cells may exhibit contact inhibition and cease production of the appropriate factors necessary to support proliferation. If the openings are too large, the stromal cells may be unable to stretch across the opening; this will also decrease stromal cell production of the appropriate factors necessary to support proliferation. When using a mesh type of framework, openings ranging from about 150 μm to about 220 μm are satisfactory. However, depending upon the three-

dimensional structure and intricacy of the framework, other sizes may work equally well. In fact, any shape or structure that allows the stromal cells to stretch and continue to replicate and grow for lengthy time periods will work in accordance with the invention. For example, for felt-type frameworks, openings ranging from about 80 μm to about 120 μm are preferred.

According to a preferred embodiment, the scaffold is a felt, which can be composed of a multifilament yarn made from a bioabsorbable material, e.g., PGA, PLA, polygluconate (PLGA) or hyaluronic acid. The yarn is made into a felt using standard textile processing techniques consisting of crimping, cutting, carding and needling. According to a further preferred embodiment, the porosity of the felt ranges from 80-98%, the density of the felt ranges from 30-60 mg/cc and the thickness of the felt ranges from 1-7 mm.

In an embodiment wherein the scaffold is made of collagen, the collagen may be in the form of a sponge, a braid or woven threads, etc. In an embodiment wherein the scaffold is made of nylon, a convenient nylon mesh is Nitex, a nylon filtration mesh having an average pore size of 210 μm and an average nylon fiber diameter of 90 μm (#3-210/36 Tetko, Inc., N.Y.).

Although Applicants are under no duty or obligation to explain the mechanism by which the invention works, a number of factors inherent in the three-dimensional framework may contribute to its successful use in the present invention:

(a) The three-dimensional framework provides a greater surface area for protein attachment, and consequently, for the adherence of stromal cells <u>in vivo</u>.

(b) Because of the three-dimensionality of the matrix, as noted above, the stromal cells that attach to the framework continue to actively grow and produce growth and regulatory factors which promote new cartilage formation <u>in vivo</u>, and are less likely to exhibit contact inhibition.

(c) The three-dimensional framework allows for a spatial distribution of cellular elements which is analogous to that found in vivo.

(d) The increase in potential volume for cell growth in the three-dimensional structure may allow the establishment of localized microenvironments conducive to cellular differentiation and maturation in the production of new cartilage in vivo.

(e) The three-dimensional matrix maximizes cell-cell interactions by allowing greater potential for movement of migratory cells, such as macrophages, monocytes and possibly lymphocytes.

(f) It has been recognized that maintenance of a differentiated cellular phenotype requires not only growth/differentiation factors but also the appropriate cellular interactions. The present invention effectively recreates the in vivo tissue microenvironment.

According to one embodiment of the invention, the scaffold may comprise or be modified, e.g., coated or impregnated, prior to implantation with certain substances to enhance the attachment and growth of chondrocytes and other stromal cells on the scaffold in vivo. These substances include, but are not limited to, bioactive agents such cellular growth factors (e.g., TGF-β), substances that stimulate chondrogenesis (e.g., BMPs that stimulate cartilage formation such as BMP-2, BMP-12 and BMP-13), factors that stimulate migration of stromal cells to the scaffold, factors that stimulate matrix deposition, anti-inflammatories (e.g., non-steroidal anti-inflammatories), immunosuppressants (e.g., cyclosporins), as well as other proteins, such as collagens, elastic fibers, reticular fibers, glycoproteins or glycosaminoglycans, such as heparin sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, keratin sulfate, etc. For example, growth factors such as TGF-β, with ascorbate, have been found to trigger chondrocyte differentiation and cartilage formation by chondrocytes. In addition, hyaluronic acid is a good substrate for the

attachment of chondrocytes and other stromal cells and can be incorporated as part of the scaffold or coated onto the scaffold.

These bioactive agents may also be included in or on the scaffold for local, sustained release of the agents. Examples of such sustained release formulations include composites comprising the bioactive agent and a biocompatible polymer, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb et al., 1992, Polymers for Advanced Technologies 3:279-292. Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), 1990, "Biodegradable Polymers as Drug Delivery Systems, Vol. 45 of Drugs and the Pharmaceutical Sciences, M. Dekker, New York.

4.2. THE PERIOSTEAL/PERICHONDRIAL TISSUE AND ITS IMPLANTATION IN COMBINATION WITH THE SCAFFOLD

The scaffold of the invention as described above is implanted into the defect site in vivo in combination with periosteal tissue, perichondrial tissue or a combination of the two tissues. Periosteal tissue is derived from the periosteum, a fibrous membrane localized at the surfaces of bones, and can be obtained from the periosteum/bone interface of any suitable bone of the patient (or subject) or a histocompatible donor, e.g., ileum, scapula, tibia, fibula, femur, etc. The periosteal tissue contains a variety of stromal cells including osteocytes, chondrocytes and fibroblasts as well as mesenchymal stem cells having the potential to differentiate into osteogenic or chondrogenic cells. Perichondrial tissue is derived from the perichondrium, the fibrous connective tissue covering cartilage, except articular surfaces. Perichondrial tissue contains chondrogenic progenitor cells and chondrocytes.

The periosteal/perichondrial tissue can be in the form of a segment or layer of tissue of any size or shape, preferably of a size and shape that fits within or corresponds to the defect site. The tissue can be laid over or under the scaffold at the implantation site and can optionally be mechanically fixed to the scaffold and/or the defect site, e.g., by sutures or glue fixation, e.g., fibrin glue. Although it may be preferable for the periosteal/perichondrial tissue to be autologous (i.e., derived from the subject receiving the implant), the tissue may be derived from a heterologous source. When periosteal/perichondrial tissue from a heterologous source is used, it may be preferable to add anti-inflammatory factors or immunosuppressants to the defect site, e.g., attached to or within the scaffold or exogenously administered to the site, to minimize the risk of immunological rejection.

According to a preferred embodiment of the invention, the three-dimensional scaffold is implanted at the defect site in vivo and a piece of periosteal/perichondrial tissue is placed over the implanted scaffold and sutured in place so that the tissue overlays and lies adjacent to the scaffold structure. Alternatively, a segment of periosteum or perichondrium may be implanted directly into the defect site and the scaffold placed on top of the tissue such that the stromal cells of the tissue can migrate from the tissue into the scaffold. In any case, the periosteal/perichondrial tissue should be situated with respect to the scaffold in such a way as to allow the stromal cells from the tissue to migrate into the scaffold and proliferate thereon and therein. The scaffold and/or periosteal/perichondrial tissue can be implanted using surgical techniques well known in the art, e.g., arthroscopy.

According to a preferred embodiment, the periosteal tissue is situated or oriented such that the cambium layer of the tissue is facing into the defect; thus, in the embodiment wherein the scaffold is placed directly into the defect site and the periosteal tissue is placed on top of the scaffold,

the periosteal tissue is oriented in relation to the top of the scaffold such that the cambium layer is facing the top of the scaffold. When perichondrial tissue is used in the claimed methods, the perichondrial tissue is also placed into the defect site or oriented with respect to the scaffold such that its cambium or inner transition layers faces the defect or scaffold. It is these layers that contain chondrogenic stem cells and/or chondrocytes that can migrate into the scaffold for the production of new cartilage at the defect site.

In an alternative embodiment of the invention, a bioresorbable patch, e.g., film, mesh or felt, can be used in place of the periosteal/perichondrial tissue and situated or oriented adjacent to the scaffold within the defect site. For example, if a film is used, it may be comprised of PGA or polygluconate; if a mesh or felt is used, they may be comprised of vicryl or PLA. The preparation of stromal cells is seeded into the defect site as described herein.

According to a further embodiment of the invention, the defect site is treated, preferably prior to implantation, to degrade the cartilage at the site of the defect, freeing cells (e.g., stromal cells) from that area to migrate into the scaffold of the implant and promoting the orderly deposition of new cartilage. When enzymes are used to treat the defect site, such enzymes include but are not limited to trypsin, chymotrypsin, collagenase, elastase, and/or hyaluronidase, Dnase, pronase, chondroitinase, etc. Alternative methods of treating the defect site to degrade the cartilage include abrasion, debridement, shaving or microdrilling. Where abrasion techniques are utilized, the surface of the cartilage may be serrated, e.g., via wire wool. In microdrilling, a drilling device is used to create small defects or channels in the cartilage. Treatment of the defect site to degrade or disrupt the pre-existing cartilage reduces the chances of scar tissue forming at the site and promotes the orderly deposition of new cartilage at the defect site.

4.3. THE STROMAL CELL PREPARATION

According to the methods of this invention, a preparation of stromal cells is additionally administered at the implantation site, which cells produce biological factors that promote chondrogenesis and the migration of cells such as chondrogenic stem cells or chondrocytes, from the *in vivo* environment adjacent to the implant, including from the periosteal/perichondrial tissue, to the scaffold for attachment and/or differentiation thereon and therein. The stromal cell preparation also provides a direct source of stromal cells, e.g., chondrocytes and/or chondrocyte progenitor cells, that are capable of migrating to the scaffold, attaching thereto, and elaborating cartilage-specific macromolecules and extracellular matrix proteins for the production of new cartilage at the defect site. The cells described herein can be administered before, during or after implantation of the scaffold and/or periosteal/ perichondrial tissue, as discussed in Section 4.4, *infra*.

The stromal cells of the preparation may include chondrocytes, chondrocyte progenitor cells including mesenchymal stem cells, fibroblasts, fibroblast-like cells and/or cells capable of producing collagen type II and other collagen types, and proteoglycans which are typically produced in cartilaginous tissues. The stromal cells can be obtained from the patient (or subject) or a histocompatible donor. The chondrocytes, progenitor cells, fibroblast-like cells and other cells and/or elements that comprise the stroma may be fetal or adult in origin, and may be derived from convenient sources such as cartilage, bone, skin, ligaments, tendons, muscles, placenta, umbilical cord, etc. For example, stromal cells such as chondrocytes may be derived from any type of cartilage, including but not limited to, hyaline cartilage, costal cartilage, fibrous cartilage, etc., which can be obtained by biopsy (where appropriate) or upon autopsy. Chondrocyte progenitor cells may be derived from various sources including bone marrow, periosteum, perichondrium or various sources of undifferentiated human